AMENDMENTS
In the Specification
Amendments to Summary of the Invention - Pages 2-5

The present invention provides a new photoreceptor/pineal gene or DNA sequence, aryl-hydrocarbon receptor interacting protein-like 1 (*AIPL1*), encoding an aryl-hydrocarbon receptor interacting protein, which maps within an LCA4 candidate region of chromosome 17p13. The protein comprises three tetratricopeptide (TPR) motifs, which are thought to impart it with nuclear transport or chaperone activity to the protein.

The present invention also provides gene sequences encoding mutant forms of the *AIPL1* gene, where the mutants forms are selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

The present invention provides a method for identifying photoreceptor/pineal-expressed gene, aryl-hydrocarbon receptor interacting protein-like 1 (*AIPL1*) including specific mutations that give rise to LCA or other retinal diseases.

The present invention also provides an anti-sense base sequence capable of binding to and allowing identification of a mutant *AIPL1* gene including anti-sense sequences for the mutants selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

The present invention also provides chip-based probes including a chip surface have attached thereto DNA anti-sense sequences having a length between about 4 and about 35 base units, where each anti-sense sequence comprises a mutation of the *AIPL1* gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

The present invention also provides a library of anti-sense DNA probes, where each probe is an anti-sense DNA sequence comprising a mutation of the *AIPL1* gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

The present invention also provides a method for screening patients including the steps of fragmenting a DNA sample from a patient into fragments of single stranded DNA, contacting the fragments with an anti-sense probe including a mutation of the *AIPL1* gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof and detecting any duplexed fragments where the duplex is formed between a fragment and the probe.

The present invention also provides a method for screening patients including the steps of fragmenting a DNA sample from a patient into fragments of single stranded DNA, contacting the fragments with an anti-sense probe comprising a mutation of the *AIPL1* gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof, amplifying any duplexed DNA, and detecting the duplexed DNA where the duplexed DNA is formed between a fragment and the probe.

The present invention also provides a method for screening patients including the steps of fragmenting a DNA sample from a patient into fragments of single stranded DNA, contacting the fragments with an anti-sense probe including a mutation of the *AIPL1* gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X,

Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof, separating any formed DNA duplexes, amplifying the duplexed DNA, and detecting the duplexed DNA where the duplex is formed between a fragment and the probe.

The present invention also provides a method for ameliorating at least one symptom of a retinal disease including administering to a retinal site an effective amount of a protein prepared from a DNA coding sequence encoding a wild-type *AIPL1* gene. The administration can by oral administration, intravenous administration intra-arterial administration, site specific administration, other similar mean of administering a gene sequence or protein or mixtures or combinations thereof.

The present invention also provides a method for ameliorating at least one symptom of a retinal disease including administering to cells of a retinal site an expression vector including a wild-type *AIPL1* coding sequence to cause the expression of a protein corresponding to the *AIPL1* coding sequence, where the protein ameliorates at least on symptom of a retinal disease.

The present invention also provides a method for identifying patients with mutations to an AIPL1 gene including the step of obtaining a DNA sample from the patient, isolating polynucleotide extracted from said sample, hybridizing a detectably labeled oligonucleotide to the isolated polynucleotide, the oligonucleotide having at its 3' end at least 15 nucleotides complementary to a wild type polynucleotide sequence having at least one mutation or polymorphism, attempting to extend the oligonucleotide at its 3'-end; ascertaining the presence or absence of a detectably labeled extended oligonucleotide; and correlating the presence or absence of a detectably labeled extended oligonucleotide in step (e) with the presence or absence of a AIPL1 mutation. The mutation are selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or

mixtures or combinations thereof; while the benign polymorphisms are selected from the group consisting of IVS1-9G->A, IVS2+66G->C, IVS2-88C->T, IVS2-14G->A, IVS2-10A->C, IVS3-25T->C, IVS3-21T->C, IVS5+18G->A, Asp90His, Phe37Phe, Ser78Ser, Cys89Cys, Leu100Leu, His172His, Pro217Pro, Asp255Asp and mixtures and combinations thereof.

Amendments to Figure 1 Description - Page 5 bridging Page 6

Figure 1 depicts a gene and protein structure of *AIPL1*; ***a.*** *AIPL1* consists of six exons, with alternate polyadenylation sites in the 3' untranslated region, shown by arrows. Cys239Arg denotes the location of the TGC→CGC missense mutation in exon 5 of the RFS128 family. Trp278X denotes the location of the TGG→TGA nonsense mutation in exon 6 of the KC, MD, RFS127 and RFS121 families. Ala336Δ2 denotes the location of the 2 bp deletion in exon 6 of RFS121. Benign coding sequence substitutions identified were Phe37Phe (TTT/TTC; 0.98/0.02 frequency), Cys89Cys (TGC/TGT; 0.99/0.01), Asp90His (GAC/CAC; 0.84/0.16), Leu100Leu (CTG/CTA; 0.57/0.43) and Pro217Pro (CCG/CCA; 0.61/0.39) ***b.*** Protein sequence of AIPL1. The alignment demonstrates the high level of sequence conservation between rat and human AIPL1, SEQ ID NO. 79 and SEQ ID NO. 1, respectively, and mouse and human AIP, SEQ ID NO. 81 and SEQ ID NO. 80, respectively. Identical residues in the four sequences are noted with an asterisk; identical residues in three of the sequences are indicated with a period.;

Amendments to Page 14 second paragraph through fourth paragraph

The present invention also provides gene sequences encoding mutant forms of the *AIPL1* gene selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

The present invention provides a method for identifying photoreceptor/pineal-expressed gene, aryl-hydrocarbon receptor interacting protein-like 1 (*AIPL1*) including specific mutations that give rise to LCA or other retinal diseases.

The present invention also provides a anti-sense base sequence capable of binding to and allow identification of a mutant *aipl1* gene including the mutants selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

Amendments to Page 15 paragraphs 1-3

The present invention also provides a library of DNA probes, where each probe is a DNA sequence including a mutation of the AIPL1 gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

The present invention also provide a method for screening patients including the steps of fragmenting a DNA sample from a patient into fragments of single stranded DNA, contacting the fragments with an anti-sense probe including a mutation of the AIPL1 gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof and detecting any duplexed fragments where the duplex is formed between a fragment and the probe.

The present invention also provide a method for screening patients including the steps of fragmenting a DNA sample from a patient into fragments of single stranded DNA, contacting the fragments with an anti-sense probe including a mutation of the AIPL1 gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82),

Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof, amplifying any duplexed DNA, and detecting the duplexed DNA where the duplexed DNA is formed between a fragment and the probe.

Amendments to Page 16 paragraph 1

The present invention also provide a method for screening patients including the steps of fragmenting a DNA sample from a patient into fragments of single stranded DNA, contacting the fragments with an anti-sense probe including a mutation of the AIPL1 gene including the mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof, separating any formed DNA duplexes, amplifying the duplexed DNA, and detecting the duplexed DNA where the duplex is formed between a fragment and the probe.

Amendments to Page 17 paragraphs 1-3

The present invention also provides a method of determining an AIPL1 mutation including the steps of detecting a mutation in a nucleic acid encoding AIPL1 protein in a sample, the mutation comprising one or more mutations selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof; and correlating detection of the mutation with a retinal disease or a propensity of pass a retinal disease to offspring.

The present invention also provides an isolated AIPL1 amino acid sequence comprising an amino acid sequence having at least one mutation, said mutation selected from the group consisting of Ala336Δ2, Trp278X, Cys239Arg, M79T, L88X, V96I, T124I, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351D12, Cys42X (TGT -> TGA), Val33ins 8 bp (GTGATCTT SEQ ID NO. 82), Leu257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

The present invention also provides an isolated nucleic acid sequence, wherein the nucleic acid encodes at least one AIPL1 mutation resulting in an amino acid substitution at any one of the following locations 336, 278, 239, 79, 88, 96, 124, 376, 163, 197, IVS2-2, 262, 302, 351D12, 42, 33 ins 8 bp (GTGATCTT SEQ ID NO. 82), 257del 9 bp (CTCCGGCAC SEQ ID NO. 83) or mixtures or combinations thereof.

Amendments to Page49 Table 1

Table 1
Intron/exon organization of *AIPL1*

Exon/ Intron	Exon Length (bp)	Starting position in cDNA[a]	Acceptor splice site[b]	Donor splice site[b]
1	96	1		CGGATCCCGAgtgagtggggccctccggagcaga SEQ ID NO. 50
2	180	97	cagagtgcaccgtctcggtgactagGTGATCTTTC SEQ ID NO. 51	CSACACCATCgtaagtaggccctgcgcgcctgtct SEQ ID NO. 52
3	189	277	gccatccatccgtttatccccacagCACACGGGGG SEQ ID NO. 53	GCTGCTGCAGgtggggctggggttggcagggctgg SEQ ID NO. 54
4	177	466	cactgacctgcagctctggggccagGTTGATGCCC SEQ ID NO. 55	GCAGACCAAGgtcagaggccgctggccacggggtg SEQ ID NO. 56
5	142	643	catggctgaccttctccctgggcagGAGAAGCCRT SEQ ID NO. 57	CACCACCCAGgtgcgcggggctgcaggggcggaca SEQ ID NO. 58
6	754/1563[c]	785	gctggatgctccctgctccccacagGCATCGTGAA SEQ ID NO. 59	

[a]Numbering based on cDNA sequence, with position 1 as the first base of the ATG start codon.
[b]The exonic and intronic sequences are represented in upper and lowercase, respectively.
[c]Lengths differ by 709 bp in the 3' untranslated region due to alternate polyadenylation signal usage.

Amendments to Page 57 Third Paragraph

PCR of a mouse retinal cDNA library using PCR primers designed to the human *AIPL1* cDNA (5'-AAGAAAACCATTCTGCACGG-3' SEQ ID NO. 42 and 5'-TGCAGCTCGTCCAGGTCCT-3' SEQ ID NO. 43) obtained a 613 bp fragment of mouse *AIPL1* cDNA. Sequencing of the resulting fragment using the AmpliCycle Sequencing kit (Perkin Elmer) and ^{32}P end-labeled primers confirmed that the resulting fragment represented mouse *Aipl1* cDNA. The fragment was used as a probe for digoxygenin *in situ* hybridization using previously described methods (Furukawa, T., Morrow, E.M. & Cepko, C.L. *Crx*, a novel *otx*-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* **91**, 531-541 (1997)).

Amendments to Page 58 First Paragraph

The Human BAC I library was screened commercially (Genome Systems) using PCR primer pairs based on the *AIPL1* sequence (5'-GACACCTCCCTTTCTCC-3' SEQ ID NO. 44 and 5'-GCTGGGGCTGCCTGGCTG-3'SEQ ID NO. 45; 5'-CCGAGTGATTACCAGAGGGA-3' SEQ ID NO. 46 and 5'-TGAGCTCCAGCACCTCATAG-3'SEQ ID NO. 47). The inventors purified BAC DNA from the identified clones using the Plasmid Midiprep Kit (QIAGEN) and sequenced it directly using an ABI310 automated sequencer. A primer walking strategy beginning with PCR primers to the cDNA obtained complete intronic sequences. The inventors viewed, edited and aligned sequence data using AutoAssembler (Perkin Elmer) software.

Amendments to Page 60 Second Paragraph

The inventors performed allele-specific PCR in RFS121 using PCR primers specific to *AIPL1* exon 6 sequence, with the forward primer annealing specifically to the wild-type sequence for codon 278 (5'-ACGCAGAGGTGTGGAATG-3' SEQ ID NO. 48) and the reverse primer in the 3' untranslated sequence (5'-AAAAAGTGACACCACGATC-3' SEQ ID NO. 49). The inventors sequenced PCR products as described above.

Amendments to Page 61 Table 5

Table 5
PCR primer sets for amplification of SSCP fragments of *AIPL1*

Amplified Fragment (Size [bp])	Primer Sequences	Size(s) Analyzed (bp)
Exon 1 (240)	5'-GGACACCTCCCTTTCTCC-3' SEQ ID NO. 60 5'-GCTGGGGCTGCCTGGCTG-3' SEQ ID NO. 61	240
Exon 2 (297)	5'-GGGCCTTGAACAGTGTGTCT-3' SEQ ID NO. 62 5'-TTTCCCGAAACACAGCAGC-3' SEQ ID NO. 63	151, 146[a]
Exon 3 (364)	5'-AGTGAGGGAGCAGGATTC-3' SEQ ID NO. 64 5'-TGCCCATGATGCCCGCTGTC-3' SEQ ID NO. 65	210, 154[a]
Exon 4 (315)	5'-TTTCGGGTCTCTGATGGG-3' SEQ ID NO. 66 5'-GCAGGCTCCCCAGAGTC-3' SEQ ID NO. 67	187, 128[a]
Exon 5 (279)	5'-GCAGCTGCCTCAGGTCATG-3' SEQ ID NO. 68 5'-GTGGGGTGGAAAGAAAAG-3' SEQ ID NO. 69	169, 110[a]
Exon 6 (497)	5'-CTGGGAAGGGAGCTGTAG-3' SEQ ID NO. 70 5'-AAAAGTGACACCACGATCC-3' SEQ ID NO. 71	273, 154, 70[a]

[a]Sizes after restriction digestion.